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Biphasic expression of slow myosin light chains and slow tropomyosin isoforms during the development of the human quadriceps muscle

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Using a two-dimensional electrophoresis technique coupled with sensitive silver staining, we have investigated the chronology of appearance of the myosin light chain and tropomyosin isoforms during early stages of human quadriceps development. Our results show that slow myosin light chains and the slow tropomyosin isoform are not detected at 6 weeks of gestation. These isoforms transiently appear between 12.5 weeks and 15 weeks of gestation and then disappear. The slow myosin light chains are re-expressed at 31 weeks of gestation and the slow tropomyosin isoform later at 36 weeks of gestation, and normally remained expressed into the adulthood. Our study thus reveals a biphasic expression of the slow myosin light chains and the slow tropomyosin isoform in developing human quadriceps muscle.

Myosin light chain; Tropomyosin; Development; Human; Muscle; Two-dimensional electrophoresis

1. INTRODUCTION

In muscle, the myosin light chains (MLC) are associated with myosin heavy chains (MHC) to form the myosin molecule. There are two major slow types (MLC1S and MLC2S) and one minor species (LC1'S) found in some mammalian slow muscles [1,2] but never seen in man, as well as three fast types (MLC1F, MLC2F and MLC3F). During muscle development there is a transient expression of an embryonic MLC (MLC1emb) [2].

Tropomyosin is a component of the contractile apparatus of sarcomeres. In striated muscle, it is involved in the calcium-dependent regulation of actin–myosin interaction. Two subunits have been described [3] called α or β according to their electrophoretic mobility. Fast and slow muscles have been shown to contain specific isoforms of tropomyosin [4,5].

A previous study [6] has demonstrated a biphasic expression of slow MHC during development of human muscle; however, concerning the expression of slow MLCs, such an observation has not been previously described [7–12]. The aim of this study was to determine the chronology of the appearance of myosin light chains during the early stages of human muscle development. We have also examined the expression of tropomyosin isoforms. In the present work, we report that slow MLC and slow tropomyosin isoforms are expressed in a biphasic manner during the development of human muscle. We therefore present evidence that a strong correlation exists between these biochemical data and the sequential appearance of myofibers.

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Abbreviations: 2D-PAGE, two-dimensional polyacrylamide gel electrophoresis; MLC, myosin light chain; MHC, myosin heavy chain; MLC1S, MLC2S, slow myosin light chains; MLC1F, MLC2F, MLC3F, fast myosin light chains; MLC1emb, embryonic myosin light chain; α_s TM, slow tropomyosin isoform; α_f TM, fast tropomyosin isoform; β TM, β tropomyosin isoform

2. EXPERIMENTAL

2.1. *Specimens*

We have studied 13 fetuses (6, 12.5, 15, 22, 22, 23, 24, 24, 25, 31, 36, 37, 37 weeks of gestation) obtained by spontaneous or therapeutic abortions and two necropsy samples from infants aged 8 months and 2 years. There was no evidence of any underlying neuromuscular disease in any of these samples; one adult biopsy (45-year-old man) was obtained during surgery. The muscle samples were always removed from the median portion of the vastus lateralis. Muscles were immediately frozen in liquid nitrogen and stored at −80°C for biochemical analysis.

2.2. *Two-dimensional gel electrophoresis*

Muscle samples weighing 20–50 mg were scissor-minced in 2 vols (w/v) of sample buffer: 9.95 M urea, 4% NP40, 2% Ampholines LKB 5–7, 100 mM DTT; 1 mg of solid urea per mg of tissue was then added. The muscles were extracted for 15–20 min at room temperature and were vortexed several times during this period. Samples were then stored either at −20 or −80°C. Prior to electrophoresis the samples were centrifuged for 5 min in a microfuge to remove any tissue fragments. 2–4 μl of the muscle extract were loaded onto the isofocusing gel. 2D-PAGE was carried out according to O'Farrell [13] as modified by Garrels [14]. In order to increase the sensitivity of detection, the proteins were silver stained as described by Morrissey [15].

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3. RESULTS AND DISCUSSION

3.1. *Biphasic expression of the slow MLCs*

At 6 weeks of gestation (Fig. 1A) the human quadriceps muscle expressed the fast MLCs, MLC1F and MLC2F, and the embryonic MLC, MLC1emb, but did not contain any slow MLCs; although this sample was the only one examined at this stage, we assume that even small amounts of these isoforms could have been detected, considering the quality of the gel obtained and the high sensitivity of the staining technique.

Between 12.5 and 15 weeks of gestation (Fig. 1B,C) there was an accumulation of slow MLCs, MLC1S and MLC2S. There was no change in the expression of fast

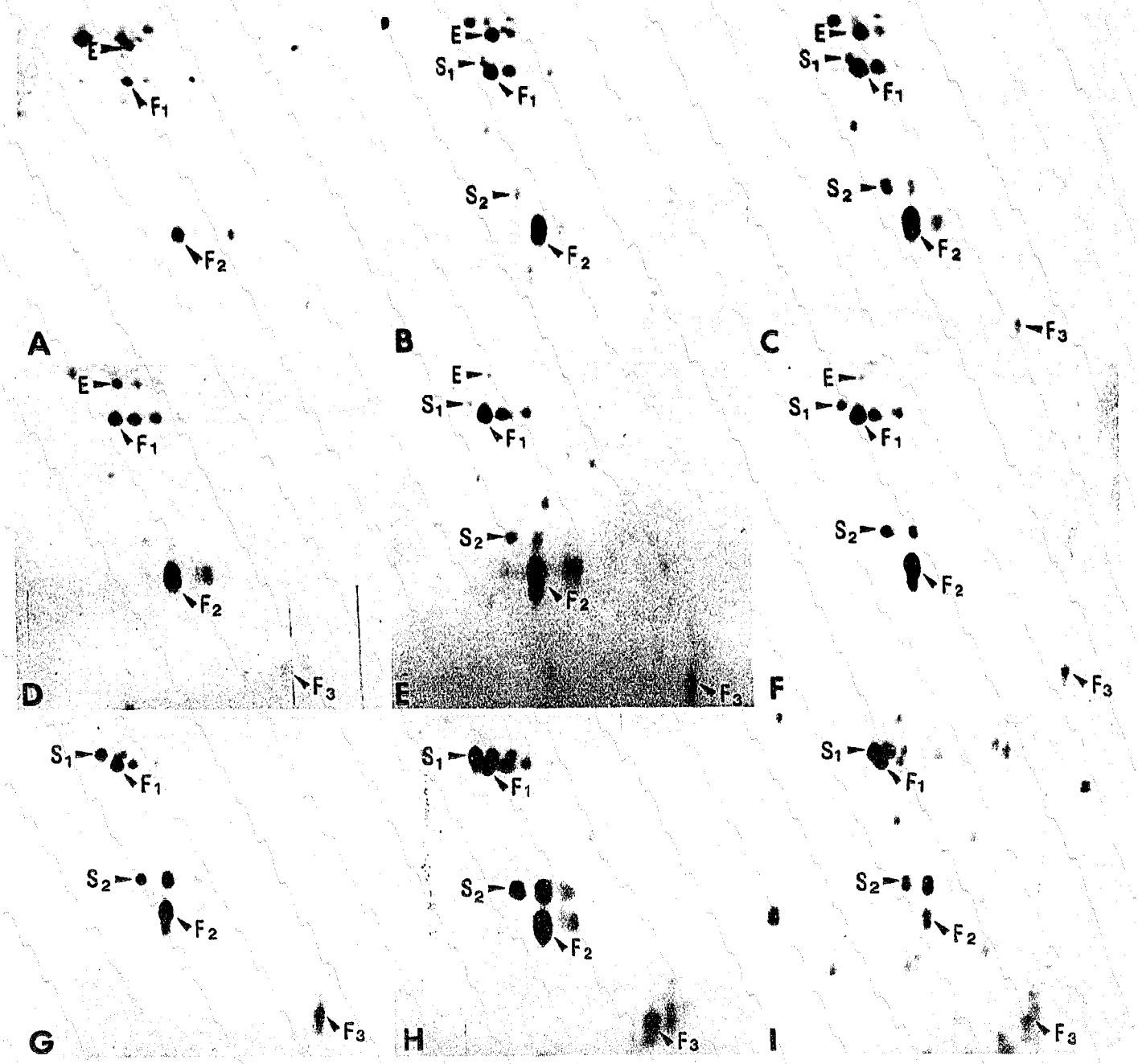

Fig. 1. Evolution of myosin light chains during development of human quadriceps muscle. 2D-PAGE of total extracts of human quadriceps muscle at 6 (A); 12.5 (B); 15 (C); 22 (D); 31 (E) and 36 (F) weeks of gestation, and 8 months (G), 2 years (H) and adult (I). The proteins were silver stained. The various myofibrillar proteins are indicated as follows: embryonic myosin light chain (E); slow myosin light chains (S1 and S2); fast myosin light chains (F1, F2 and F3).

MLCs; however, the amount of MLC1emb increased and we could already detect the appearance of the fast MLC, MLC3F at 15 weeks of gestation.

The finding that slow myosin light chains are present in early development of human muscle has never been observed in previous studies [7-10,12], except for MLC2S that has been detected once at 10 weeks [11]. In early stages of development, slow myosin constitutes a very small proportion of the total myosin detected by electrophoresis [16]. In our study, because of the increased sensitivity of the silver staining technique, we have been able to detect the expression of slow MLCs as early as 12.5 weeks. It was also possible to detect MLC3F by the age of 15 weeks of gestation. This isoform has been reported to be present at 10 weeks in a recent study [11], but was never detected before 25 weeks of gestation by other authors [7-10,12].

By 15 weeks of gestation we started to detect some additional acidic spots related to MLC1F, MLC2F and MLC2S. This polymorphism has been already reported to be phosphorylated in phosphorylatable isoforms in the case of MLC2 [9,11]. However, we currently have no explanation concerning the polymorphism of MLC1F which belongs to the non-phosphorylatable class of myosin light chains, although this phenomenon has been observed in the results presented by others [9,11].

At 22 weeks of gestation (Fig. 1D), the slow MLCs were almost undetectable whereas the fast MLCs were still present and the amount of MLC1emb slightly decreased. It is interesting to note that the slow MLCs never completely disappear. This persistence of slow MLCs could be related to a finding of previous authors [6], who observed a persistence of the slow myosin heavy chain in the large Wohlfart type B fibers [17] in developing human skeletal muscle at 20 weeks of gestation. By 31 weeks of gestation (Fig. 1E), the amount of slow MLCs increased significantly, the three fast myosin light chains were still present and a trace of MLC1emb was still visible. This second increase in accumulation of slow MLCs corresponds to that reported to occur after 30 weeks of gestation [7-10,12].

By 36 weeks of gestation (Fig. 1F), all the adult fast and slow isoforms were present, and only a small amount of MLC1emb could still be detected; this MLC was completely eliminated by 37 weeks of gestation (data not shown). This latter observation differs slightly from results of previous studies [9,10,12] where MLC1emb was not detected after 33 weeks of gestation. Nevertheless, the evolution of the ratio MLC1emb/MLC1F which decreased throughout the development, was very well correlated to the finding that MLC1emb is gradually replaced by MLC1F [18]. At 8 months and 2 years after birth (Fig. 1G,H), all the fast and slow

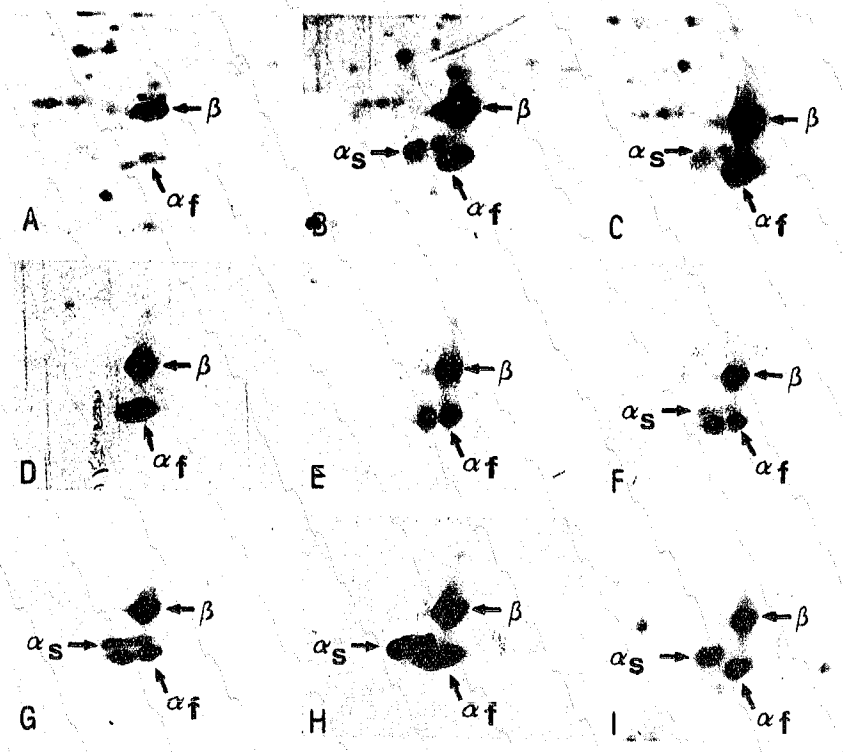

Fig. 2. Evolution of tropomyosin isoforms during development of human quadriceps muscle. 2D-PAGE of total extracts of human quadriceps muscle at 6 (A); 12.5 (B); 15 (C); 22 (D); 31 (E) and 36 (F) weeks of gestation, and 8 months (G), 2 years (H) and adult (I). The proteins were silver stained. The different isoforms of tropomyosin are indicated as follows: α_f = fast isoform; α_s = slow isoform; $\beta = \beta$ isoform.

MLCs were clearly visible so that the pattern of contractile proteins was identical to that found in the adult quadriceps (Fig. 1I).

3.2. *Biphasic expression of slow tropomyosin isoform*

We have also looked at the expression of tropomyosin isoforms during the development of quadriceps muscle (Fig. 2). At an early stage of development (6 weeks of gestation), the β TM was the major subunit detected while a small amount of α_f TM was also present (Fig. 2A). The predominance of the β TM in embryonic muscle has been previously reported in chicken and rabbit [4,19]. Our finding indicates that in man, the β TM is also the major subunit present in embryonic muscle. Between 12.5 and 15 weeks of gestation, a significant amount of α_s TM was detected, whereas α_f TM and β TM were expressed in almost equal amounts (Fig. 2B,C). Other studies on the developmental expression of tropomyosin isoforms have demonstrated the early expression of α_s TM, in the 11–15-day-old chick embryo, and in rabbit striated muscle [4,19]. Our study extends this observation to the human species. Between 22 and 31 weeks of gestation, the major change was the disappearance of α_s TM whereas α_f TM and β TM were predominant and present in equivalent amounts (Fig. 2D,E). By 36 weeks of gestation, α_s TM reappeared (Fig. 2F) and gradually accumulated after birth (Fig. 2G,H). In the adult, similar amounts of α_s TM and α_f TM were detected (Fig. 2I).

3.3. *General conclusions*

In this study we show that fast myosin light chains and the α_f isoform of tropomyosin are expressed at all stages of the development and that not only the slow MHC [6], but also the slow MLC and tropomyosin isoforms, (i) are expressed at early stages of human muscle development, and (ii) are expressed in a biphasic manner during development. The development of muscle fibers is a complex phenomenon involving several generations of myotubes with distinct fiber rates [6,20,21]. Each of them expresses the different myosin isoforms in variable amounts, depending on the number of myotubes (primary, secondary, tertiary) that are present at a given time. It seems likely that different populations of myofibers appear at specific times of development, and that for each population there is a corresponding set of some specific myosin isoforms. Our results present evidence of such a correlation; indeed, the first accumulation of slow myosin that we detect, occurs at the same time as the appearance of primary myotubes, while the second expression corresponds to the genesis of secondary myotubes [6]. At these times, the number of primary myotubes is decreasing and the secondary myotubes are not yet completely formed, thus none or very low amounts of slow myosin and tropomyosin isoforms is detected.

Although the initial induction and subsequent disappearance of both slow isoforms (MLC and α_s TM) occur simultaneously during the early stages of development (12.5–15 weeks of gestation), the delay observed for the reappearance of α_s TM indicates that the tropomyosin subunit pattern characteristic of the adult human muscle is reached later than that of the myosin subunits. This observation leads us to conclude that the accumulation of slow myosin and tropomyosin isoforms is not strictly co-ordinated.

It would be interesting therefore to look for a correlation between the protein and mRNA levels for MLCs and tropomyosin subunits during human muscle development. It would thus be possible to know whether the regulation is due to transcriptional control in early development, and may be due to additional factors during the late fetal stage. Such studies have been described in developing fast chick muscle [22,23].

The early expression of slow myosin has been described for several species [4,21,24–28]. In view of our results, it is now possible to extend this observation to the human species. Our findings and those of others [6], suggest that there may be a specific regulation of slow myosin (light and heavy chains) and the slow tropomyosin isoform during human muscle development. More knowledge about this regulation would be very useful to understand mechanisms of muscle diseases where the expression of slow myosin isoforms is preferentially affected (unpublished results, [29]).

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